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Review

Large volume injection techniques in capillary gas chromatography

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Abstract

Large volume injection (LVI) is a prerequisite of modern gas chromatographic (GC) analysis, especially when trace sample components have to be determined at very low concentration levels. Injection of larger than usual sample volumes increases sensitivity and/or reduces (or even eliminates) the need for extract concentration steps. Also, an LVI technique can serve as an interface for on-line connection of GC with a sample preparation step or with liquid chromatography. This article reviews the currently available LVI techniques, including basic approaches to their optimization and important real-world applications. The most common LVI methods are on-column and programmed temperature vaporization (PTV) in solvent split mode. Newer techniques discussed in this article include direct sample introduction (DSI), splitless overflow, at-column, and "through oven transfer adsorption desorption" (TOTAD).
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Keywords: Gas chromatography; Injection techniques; Large volume injection; PTV; On-column; Direct sample introduction; Splitless overflow; At-column; TOTAD

Contents

1. Introduction

Modern analytical chemistry is being directed to smaller sample size, simpler sample preparation, and better sensitivity. Ultimately, full automation from sample preparation to analyte detection is desired. These trends are also obvious in modern capillary gas chromatography (GC). In trace component analysis, large volume injection (LVI) in GC constitutes one of the needed conditions to fulfill these goals. For example, injecting larger than usual volumes of final sample extracts increases method sensitivity and/or reduces the need for extract concentration steps. Also, an LVI technique can serve as an interface for on-line connection of GC with a sample preparation step, such as solid-phase extraction (SPE), or with liquid chromatography (LC) for enhanced separations using two-dimensional LC–GC or LC × GC approaches [1,2].

There are four basic types of injection techniques: isothermal (hot) split and splitless, on-column, and programmed temperature vaporization (PTV). The isothermal split and splitless

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injections are performed in the same inlet called split/splitless inlet. This split/splitless inlet is most common because of its simplicity and robustness. However, the classical splitless injection enables only 1–2 μl of a liquid onto capillary columns in most cases. Depending on the solvent, this injection can be increased up to 5–10 μl using a pressure pulse during the sample introduction process [3–5].

For the injection of large volumes up to hundreds of microliters of sample, on-column and PTV injection techniques have been mainly used (and/or modified). The most critical problem in LVI is a huge solvent vapor volume resulting from the expansion of the large liquid volume of the injected solvent. On-column injection solves this problem using a retention gap, which provides room for the large injected solvent volume to condense and expand. The PTV injection separates solvent vapor from analytes through venting of the vapor in the liner. The first PTV LVI was introduced by Vogt et al. in 1979 [6], and the on-column LVI was introduced by Grob et al. in 1985 [7]. Although at the beginning, PTV injections were employed mainly to reduce discrimination of certain analytes (as compared to classical split/splitless injection) [8], it was later considered a major alternative to on-column LVI for introduction of larger sample volumes. The on-column LVI is useful for most analytes from highly volatiles to low-volatiles, even thermally labile compounds. However, this method is not rugged for dirty samples. PTV-based LVI provides more ruggedness for dirtier extract injections [9], but it is less appropriate for highly volatile and thermally labile analytes [10].

There are several review papers that marginally deal with LVI [1,10–16]. However, only three articles reviewed LVI as a major topic in the past [17–19], two of them describing PTV-based LVI. Since the last review papers, there have been several new developments further improving LVI techniques. In this paper, we review the currently available LVI methods, including basic approaches to their optimization and most important applications.

2. On-column LVI

A key component of the on-column LVI technique is a retention gap (a piece of a deactivated, uncoated capillary column), which is attached to the front of the analytical column to retain the injected liquid. The solvent containing the analyte is introduced to the retention gap at a temperature below the solvent boiling point. The liquid spreads to the retention gap, forming a flooded zone with the solutes distributed throughout the sample layer. The solvent starts evaporating at the rear end of the flooded zone. Other volatile compounds also evaporate but they are trapped again in the liquid layer ahead. Less volatiles do not evaporate but spread out over the surface of the retention gap. These compounds are refocused by the stationary phase focusing effect. As the last portion of the solvent is evaporated, the solutes start the chromatographic process when the oven heats. This is a basic cold on-column LVI, conventional retention gap technique [7]. The maximum injection volume depends on the size of the retention gap. For instance, a 15 m × 0.32 mm I.D. retention gap enables up to 150 μl injection volume using this technique [12].

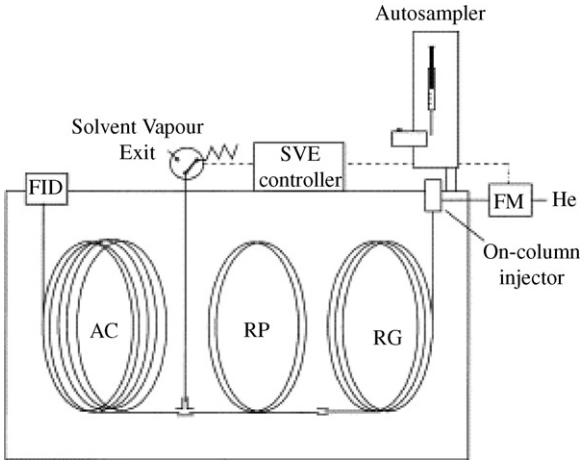

Fig. 1. Set up of the on-column large volume injection-GC system. Abbreviations: AC, analytical column; RP, retaining precolumn; RG, retention gap; FM, flow meter; He, helium; SVE, solvent vapor exit (reprinted from ref. [30] with permission from the publisher).

To increase the injection volume or shorten the retention gap, the partially concurrent solvent evaporation (PCSE) technique can be employed. In PCSE, the major part of the solvent (about 90%) is evaporated during the introduction while the remaining solvent forms the flooded zone in the retention gap [20]. This method requires speed-controlled sample introduction. The sample introduction rate should be higher than the solvent evaporation rate to keep the solvent film in the retention gap [20]. The maximum injection volume and the length of the retention gap can be calculated to prevent spreading of the solvent film to the stationary phase of the analytical column (see the next section describing the on-column LVI optimization). Generally, the PCSE on-column injection technique enables sample volume up to 800 μl [12].

In on-column LVI, solvent peaks can be too broad to be tolerated by some GC detectors. This problem was solved by setting up an early solvent vapor exit (SVE) immediately after the retention gap [21]. The SVE accelerates evaporation rate and protects the detector from excessive solvent vapor. Fig. 1 shows a typical on-column LVI with SVE. During sample introduction, the SVE is opened to discharge the solvent vapor before it enters the analytical column. The SVE is closed when the evaporation is complete. SVE enables even larger sample volume introduction than PCSE. However, on-column LVI with SVE does not behave as the classical on-column LVI described earlier. Using the SVE, highly volatile analytes can be easily lost by concurrent evaporation with the solvent. Boselli et al. investigated the solvent evaporation process in this system. They found that the solvent evaporation rate became the same as the solvent introduction rate when the SVE was opened due to the pressure drop over the flooded zone. This fully concurrent solvent evaporation prevents the solvent trapping effect, resulting in loss of high volatiles [22]. To reduce the loss of high volatiles, several methods were suggested. One method employs co-solvent trapping, *i.e.* addition of a small portion of a solvent with higher boiling point, which

is effective in some applications, such as in the case of loop type sample introduction using a less volatile pre-solvent [23,24]. In some cases, the same solvent as the sample introduction solvent can be successfully used as a presolvent [25]. Another approach involves installation of a capillary restriction column (with an I.D. smaller than the retention gap, *e.g.* 0.32 mm I.D.) between the retention gap (0.53 mm I.D.) and the SVE, which restricts the evaporation rate (see Fig. 1) [26,27]. The same effect can be also achieved by installing a narrow bore vapor outlet line [26]. In another method, the SVE is closed before the end of solvent evaporation in order to facilitate solvent trapping of the volatile components [28]. In this approach, determining the closure time of the SVE is very critical. The closure time can be derived from comparing recoveries of high volatiles at different SVE closure times [29]. This requires tedious optimization and re-optimization when configuration of the injection method is changed. To avoid this optimization process, it was suggested to monitor the effluent leaving the SVE by flame ionization detection (FID) to determine the SVE closure time. Later, Brinkman's group developed an electronic flow meter, "SVE controller", which monitors the carrier gas flow rate and closes the SVE automatically [30,31].

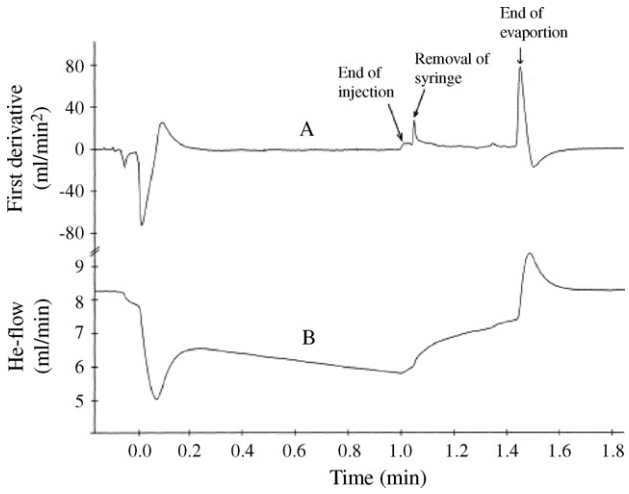

Fig. 2. Helium flow-rate (B) and its first derivative (A) for a 60-μl injection of *n*-hexane in a 6 m × 0.32 mm retention gap connected to a 1.5 m × 0.32 mm restriction column. Injection started at 0 min, needle inserted into injector 0.05 min before start of injection, and removed 0.05 min after end of injection; SVE left open all the time (reprinted from ref. [30] with permission from the publisher).

2.1. Optimization of on-column LVI

The typical on-column LVI system consists of an uncoated retention gap and the SVE with or without a restriction column behind the retention gap. Two parameters, the SVE closure time and injection rate, should be carefully optimized to achieve optimum LVI conditions in this system [31]. As we explained earlier, the SVE should not be closed too late [21] and the injection rate should be faster than the evaporation rate to keep the solvent film in the retention gap [7,20].

To optimize the injection rate, the evaporation rate should be calculated first. Hankemeier et al. investigated several methods to determine the evaporation rate [31]. They found that the best approach was to determine the evaporation rate from a plot of evaporation time *vs.* injection time at constant injection speed, injection temperature, and head pressure. Then, the injection rate, v_{inj} (μl/min), can be calculated from the Eq. (1):

$$v_{inj} = \frac{v_{evap}}{1 - (f \times L_{RG}/V_{inj} \times FZ)} \quad (1)$$

where v_{evap} (μl/min) is the evaporation rate, f is the ratio of the solvent film length *vs.* the length of the retention gap, L_{RG} (cm) is the length of the retention gap, V_{inj} (μl) is the injection volume, and FZ (cm/μl) is the flooded zone. To make sure that long enough solvent film is formed, the authors suggest $f = 0.6$. Table 1 gives examples of determination of minimum L_{RG} or maximum V_{inj} for otherwise given conditions ($f = 1$ in these extreme examples because they consider a fully flooded retention gap) [16]. Re-optimization caused by a minor change, such as replacement of the retention gap, can be conducted by adjusting the evaporation rate *vs.* the injection rate by variation of the injection temperature at a constant injection speed [31].

As explained earlier, the closure time of the SVE is very critical to prevent loss of high volatiles. The evaporation rate during injection can be monitored by the carrier gas flow rate from the SVE. The carrier gas flow sharply decreases at the start of the injection and sharply increases at the end of evaporation (see Fig. 2) [30]. The first derivative of the carrier gas flow shows the increase at the end of evaporation more clearly. Because of that, the electronic SVE controller uses the first derivative as a signal.

Table 1
Examples of determination of minimum retention gap length (L_{RG}) or maximum injection volume (V_{inj}) in the on-column LVI technique

Minimum L_{RG} for 100 μl injection?		Maximum V_{inj} for a 150 cm retention gap?	
Evaporation rate (v_{evp})	55 μl/min	Evaporation rate (v_{evp})	55 μl/min
Injection speed (v_{inj})	120 μl/min	Injection speed (v_{inj})	60 μl/min
Flooded zone (FZ)	10 cm/μl[a]	Flooded zone (FZ)	20 cm/μl[b]
Residual liquid per min ($v_{inj} - v_{evp}$)	65 μl/min	Residual liquid per min ($v_{inj} - v_{evp}$)	5 μl/min
$L_{RG} = \frac{(v_{evap} - v_{inj}) \times V_{inj} \times FZ}{f \times v_{inj}} = 540$ cm		$V_{inj} = \frac{L_{RG} \times f \times v_{inj}}{FZ \times (v_{evap} - v_{inj})} = 90$ μl	

A fully flooded retention gap is considered in these extreme examples, thus $f = 1$ in Eq. (1). Modified from ref. [16] with permission from the publisher.

[a] Typical value for a 0.53 mm I.D. retention gap.

[b] Typical value for a 0.32 mm I.D. retention gap.

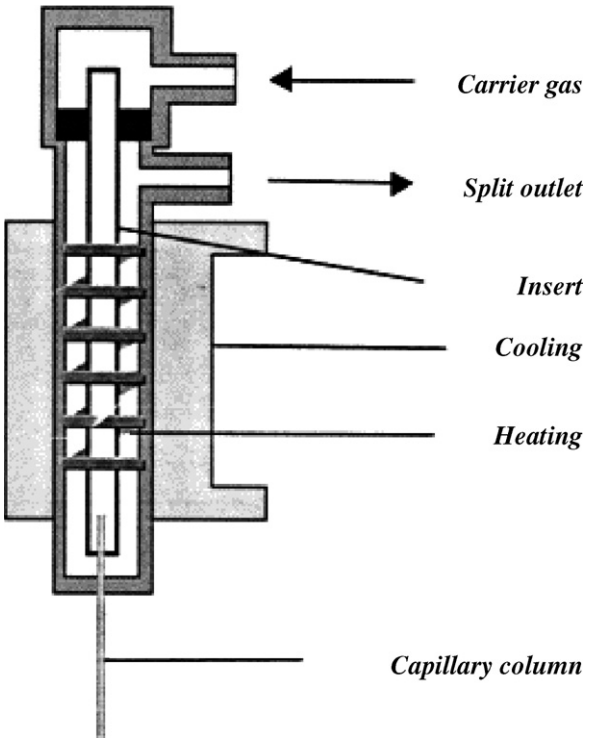

Fig. 3. Schematic diagram of the PTV (reprinted from ref. [11] with permission from the publisher).

Experiments show that a wide retention gap (0.53 mm I.D.) is preferred to a narrower retention gap (*e.g.* 0.32 mm I.D.), in which the change of the evaporation rate during injection is more significant [30]. When a narrower restriction column (0.32 mm I.D.) is attached to a 0.53 mm I.D. retention gap, the SVE should be closed at the end of the evaporation (*i.e.* automatically on a signal from the SVE controller) [26]. Without the restriction column, the optimum SVE closure time should be about 1 s earlier than the end of the solvent evaporation [27,28].

3. PTV LVI

The PTV injector is similar to a conventional split/splitless injector. In both systems, the sample is injected to a liner (insert) placed inside a vaporizer and evaporated there (see Fig. 3). However, the PTV injector is equipped with a very sophisticated temperature control function. The PTV injector can be rapidly heated or cooled during injection while the conventional split/splitless injector is isothermal. Because of this temperature control, PTV has become the most popular LVI interface.

LVI with PTV can be achieved in various modes, including splitless injection, vapor overflow, and solvent-split [11]. Among these, solvent-split injection has been the most widely used PTV technique in LVI applications. A brief principle of the solvent-split injection is as follows: (1) sample is introduced at a relatively low temperature (below solvent boiling point); (2) solvent is eliminated via split exit while the higher-boiling analytes are retained in the liner; (3) the PTV is rapidly heated and the retained analytes are transferred to the analytical column in splitless mode, keeping the oven temperature below solvent boiling temperature to refocus the analytes at the front of the column; (4) after the splitless transfer, the split exit is reopened to remove residual solvent vapor and low-volatile matrix compounds from the inlet.

The sample introduction to a vaporizer can be carried out by three approaches: at once (one injection), multiple, and speed-controlled injections. "At once injection" refers simply to one rapid injection (within 1–2 s), and the process of the injection with solvent elimination can be repeated several times ("multiple injection") to increase sample volume. The maximum injection volume in "at once injection" is given mainly by the selection of the liner size and packing material; see Table 2 for guidelines suggested by Mol et al. [18]. The next section on PTV LVI optimization discusses liners and packing materials in a more detail. Using speed programmable autosamplers, multiple injection can be replaced by a continuous process, in which the sample injection rate can be controlled to be equal to the solvent elimination rate [32]. In this way, the injection volume can be increased almost unlimitedly [16].

3.1. Optimization of PTV LVI

As previously explained, the injection volume can be increased by employing multiple or speed-controlled injections. The multiple injection technique uses the same configuration as the at once injection approach. The speed-controlled injection requires pre-determination of sample introduction rate, which is equal to the solvent elimination rate [33]. The theoretical solvent elimination rate can be calculated for different solvents by Eq. (2):

$$V_{\text{inj. max}} = V_{\text{el}} = \frac{Mp_{\text{a}}}{\rho RT_{\text{o}}} \frac{p_{\text{o}}}{p_{\text{i}}} V_{\text{t,o}} \quad (2)$$

Table 2
Guidelines for the selection of liner and injection mode in large volume PTV injection (reprinted from ref. [18] with permission from the publisher)

	Analyte		
	Very labile/adsorptive	Other	
Sample volume (μl)	>5	<100–150	>100–150
Liner I.D. (mm)	1	4	1–4
Packing	Empty	*e.g.*, Supelcoport, Chromosorb-750 coated with Dexsil	
Injection mode	Repetitive ($n \times 5$ μl) or speed controlled	At-once	Repetitive ($n \times V_{\text{max}}$) or speed controlled
Volatility application range	$\geq C_{13}$	$\geq C_9$	$\geq C_{13}$, C_{10-30}[a]

[a] When packing material is Tenax.

where $V_{inj.max}$ (μl/min) is the maximum speed of sample introduction, V_{el} (μl/min) is the solvent elimination rate, M (kg/mol) is the molar weight of the solvent, p_a (Pa) is the partial pressure of the solvent, ρ (kg/m^3) is the solvent density, R (Pa m^3 mol^{-1} K^{-1}) is the universal gas constant, T_o (K) is the outlet temperature, p_o (Pa) is the outlet pressure, p_i is the inlet pressure, $V_{t,o}$ (μl/min) is the total gas flow rate at outlet conditions (T_o and p_o). Eq. (2) indicates that the solvent elimination rate can be increased by increasing the purge gas flow-rate, reducing the pressure in the liner, and/or increasing the injection temperature, which increases p_a [33].

This theoretical equation assumes saturation of the carrier (purge) gas with solvent vapor and an isothermal evaporation process. However in reality, maximum solvent elimination rate is affected by the cooling effect from solvent evaporation. Therefore, smaller sample introduction rate than the theoretical value should be used and optimized in practice. The cooling effect is more pronounced at higher solvent evaporation rates, thus when using more volatile solvents or higher inlet temperatures during the solvent elimination [14,34]. As for the purge gas saturation with solvent vapors, it can be improved by the use of liners with a large inner surface. Therefore, liners with a suitable packing provide more effective solvent elimination than empty liners [33].

Despite the possibility of very large volumes in the speed-controlled injection technique, at once injection is more common and preferred due to its simplicity. In the original PTV injection, liners with a small I.D. are used because they reduce the residence time of the analytes in the inlet, preventing thermal degradation of the labile analytes. However, the narrow liners retain less liquid volume than wider liners of the same length. Thus, to maximize the sample injection volume with at once injection, choice of liners and packing materials as discussed below is very critical. The maximum injection volume can be determined by injecting a certain volume of solvent to a liner without column attachment but with the carrier gas flow on. The injection volume can be increased until no solvent drop is observed in the exit of the liner [35]. A wider packed liner is an obvious option to inject larger volumes.

In addition, packing materials in the liner help to retain liquid and trap high volatiles efficiently, which reduces loss of high volatiles via split exit with solvent elimination [11]. However, packing materials can cause negative effects, such as degradation of analytes, strong or even irreversible retention of analytes due to their strong affinity towards the packing material, carry over, and limitations in the heating temperature. Packing materials have different nature based on their composition [18]. For example, some of them consist of silica (glass or fused-silica wool, glass beads) that retains water strongly. Organic polymers such as Tenax or cross-linked polystyrenes resist water but strongly retain the solutes [36–38]. This strong retention may be useful for high volatiles, but causes difficulties during the transfer of high boiling compounds to the column.

Carbofrit (a carbon material) releases most high boiling compounds easily but it has strong affinity to polyaromatic compounds. Polyimide coating on glass liners packed with Carbofrit was tested by Grob et al. to protect Carbofrit destruction (to bind loose particles and hold the packing in place) and to deactivate the glass surface [39]. Mol et al. found that PTFE wool was inert, whereas polyimide wool was more adsorptive to organophosphorous pesticides [40]. Saito et al. tested four different packing materials, phenylmethylsilicone chemically bonded silica (PMSS) with Dexsil 300, SE-52/wool, and Tenax TA for the multiclass pesticide analysis in vegetables and fruits [41]. The best result was observed with PMSS as the packing material in the liner. The choice of packing material depends on each particular analysis case. Therefore, testing with targeted analytes and sample matrix are recommended on selected packing materials and liners prior to the analysis using the PTV LVI. For example, Tollbäck et al. tested four different liners: single-baffled, single-baffled with glass wool, multi-baffled, and sintered glass liner for the analysis of polybrominated diphenyl ethers (PBDEs) with PTV LVI [42]. They found the multi-baffled liner to be the best in terms of inertness and recovery.

In spite of predetermination of an optimal liner and packing, there are still limitations in some applications. For example, trapping of highly volatile compounds might still be low or peak distortions occur. These problems can be solved by injecting a co-solvent (a higher boiling point solvent) and using a retention gap [14,43,44].

After selection of the liner and packing material, the PTV parameters affecting the solvent vaporization and the transfer of the analytes to the column should be optimized to maximize recovery of the analytes. This process can be done by comparing peak areas of the analytes at different conditions. Fig. 4 shows plots of various parameters *vs.* time during a typical PTV solvent split LVI and subsequent GC run [45]. The optimization of the parameters can be accomplished either one factor at a time or by simultaneous variation of different factors using statistical design of experiments (DOE). In one factor at a time approach, the parameters for the analyte transfer phase can be determined first using PTV cold-splitless injection of a small sample volume without solvent venting. Then, the solvent vaporization parameters in PTV solvent-vent LVI can be optimized using the determined transfer conditions [35,46]. For pesticide residue analysis, Godula et al. initially optimized five parameters

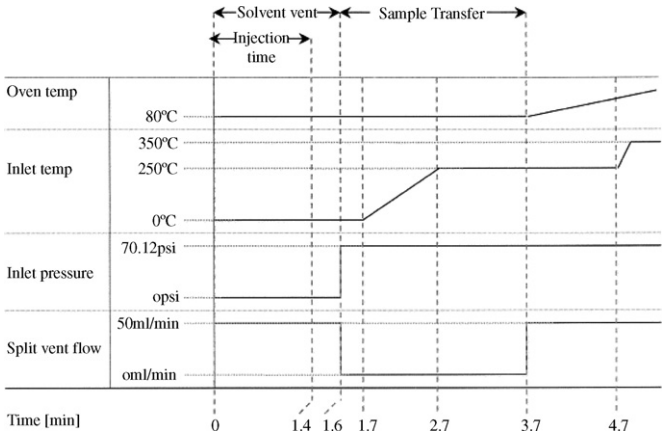

Fig. 4. Plots of various parameters *vs.* time during a typical PTV solvent split LVI and subsequent GC run (reprinted from ref. [45] with permission from the publisher).

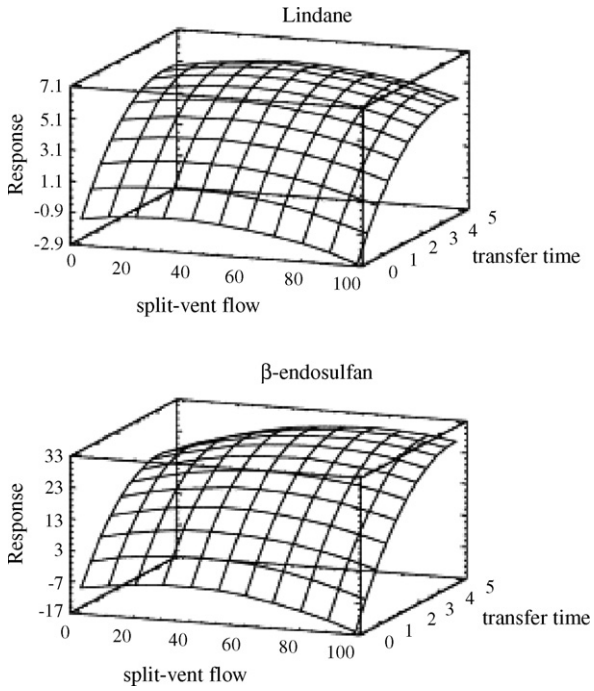

Fig. 5. Examples of application of the response surface model to optimize PTV LVI parameters; estimated response surface obtained for pesticides lindane and β-endosulfan (reprinted from ref. [47] with permission from the publisher).

(initial inlet temperature, inlet heating rate, final inlet temperature, splitless time, and initial oven temperature), followed by the optimization of vent flow (split gas flow), vent pressure, and vent time [35].

The statistical DOE can screen several factors together and determine significant factors. The significant factors are optimized by the statistical DOE, using for example full factorial design. The results are plotted as response surfaces (see Fig. 5) [47–50], allowing determination of optimum values for each optimized parameter. This statistical DOE has been applied in several studies for PTV LVI optimization. Generally, significant factors include solvent venting temperature, solvent evaporation time, split flow rate, and the splitless time (transfer time) [51]. Some general rules for PTV LVI operation can be drawn from previous studies, but experimental optimization of the PTV LVI parameters is still required for each particular case.

4. Novel injection techniques for LVI

Both the on-column and PTV LVI techniques have their advantages and limitations. On-column LVI is superior to PTV LVI for the analysis of thermally labile compounds and high volatiles. However, similarly to classical on-column injection, its tolerance for dirty or complex matrices is very low because the entire sample is injected onto the column. Therefore, it is unavoidable to change the retention gap regularly. In addition, this direct injection to column (or a retention gap) does not allow certain applications, such as direct water sample injection. Also, the on-column LVI requires a sophisticated autosampler to control injection rate precisely to prevent flooding of the system.

The PTV LVI is more robust for dirty matrix samples because the sample is injected into a liner and the sample transfer to column is performed by a vaporizer. However, thermally labile compounds may degrade in the hot liner and heavy compounds are not transferred to the column efficiently. In addition, high volatiles can be lost with solvent venting. Even though these shortcomings can be reduced by thorough optimization (which we introduced previously), the optimization process is time consuming and tedious. In addition, even a carefully chosen and deactivated packing material may cause analyte degradation and/or irreversible adsorption after several injections due to its mechanical damage and deposited non-volatile matrix components. Therefore, the packing material or liners should be replaced regularly in routine analysis.

Continuous investigation for robust, easy, and flexible LVI techniques has modified the existent LVI approaches and some new concepts have been invented [3,14,52–64]. For example, Grob and his coworkers combined PTV and on-column LVI concepts and made a vaporizer with a retention gap and SVE for LVI [14,52]. This system keeps the GC column cleaner compared to on-column LVI and reduces the loss of volatiles compared to PTV LVI. Other interesting novel LVI approaches, including direct sample introduction, splitless overflow, at-column, and "through oven transfer adsorption desorption" (TOTAD), are discussed below.

4.1. Direct sample introduction

The direct sample introduction (DSI) technique was introduced by Amirav and his coworkers [59,65]. In DSI, a liquid (or solid) sample is placed in a disposable microvial. After this step, the microvial is introduced into a PTV injection port using a manual probe (see Fig. 6) or an autosampler. In the automated version, called DMI (difficult matrix injection), the liquid sample is injected into the microvial placed in a liner, which is then inserted into the inlet (or, in a very recently introduced system, into a thermodesorption unit attached to the inlet) using a robotic arm of the autosampler [66–69].

Similarly to normal PTV solvent-split technique, the solvent evaporates through split vent, then the inlet is rapidly heated and analytes transferred in the splitless mode to the column for a GC separation. When the GC run is completed, the microvial is removed from the system and discarded along with non-volatile matrix components that remain on its inner surface. This prevents build-up of non-volatile contamination in the GC system, greatly reducing need for frequent system maintenance and sample clean-up.

The optimization of DSI/DMI is very similar to the PTV solvent-split optimization. However, the use of a microvial brings several distinct features. The liquid sample is held in the microvial, thus it does not need to be trapped in the inlet using low temperatures (cryofocusing) and/or packing materials. The maximum injection volume is limited by the size of the microvial (up to about 30 μl is possible in the current systems). The solvent evaporation takes generally longer because of the relatively small evaporation surface in the narrow microvials.

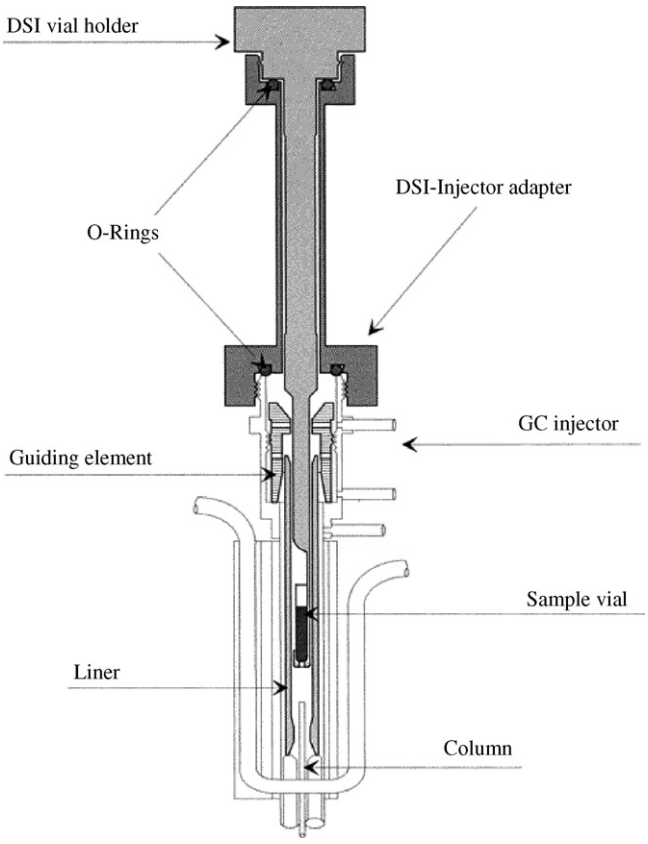

Fig. 6. Drawing of the ChromatoProbe used in DSI (reprinted from ref. [59] with permission from the publisher).

Also, the microvial increases glass surface area in the inlet and the inlet activity may vary throughout the GC sequence because a new (different) microvial (and liner) is introduced into the system each time. Recently, a unique concept of analyte protectants has been introduced in the GC analysis of pesticide residues in foods [70,71]. Analyte protectants are compounds that strongly interact with active sites in the GC system, thus decreasing degradation and/or adsorption of susceptible analytes. It was demonstrated that the addition of a suitable combination of analyte protectants to the injected samples provides effective system deactivation using classical splitless injection [71] as well as DMI [69].

4.2. Splitless overflow

Almost 40 years after its invention, classical splitless injection is still the most widely used technique of sample introduction to GC due to its simplicity. In the classical splitless injection, the sample is rapidly vaporized in the liner placed in the hot chamber and the vapors are transferred to the column by the carrier gas. The vapors are stored in the liner until the transfer is complete, thus the sample volume is limited by the liner internal volume [72]. The typical sample introduction volume through the classical splitless injection is 1–2 μl of liquid, although it can be larger depending on the liner size, solvent type, injection temperature, and inlet pressure. Using these parameters, the maximum sample volume can be calculated (*e.g.* using a freely downloadable software tool from Agilent [73]). The vapor volume formed in the liner can be reduced by the programmed elevation of column head pressure in so called pulsed splitless injection, enabling introduction of larger sample volumes of up to ~5–10 μl. Another benefit of the pulsed splitless injection involves reduction of analyte residence time in the hot injection port, resulting in significantly lower analyte degradation and/or adsorption in the inlet [3–5].

To inject even larger sample volumes using a conventional splitless injector, several splitless injection methods were developed, such as splitless overflow injection, slow injection mode or solvent diversion column [74]. However, their application is limited. Recently, Grob and Biedermann carefully examined sample evaporation in the hot vaporizer (splitless injector) and classified two injection types: thermospray and band formation [75–77]. They suggested that users can choose either injection type by optimization of the injection according to their application.

Magni and Porzano found that 20–50 μl of liquid sample can be injected successfully in splitless injection with band formation [54]. The concept of this concurrent solvent recondensation large sample volume splitless injection (CRS-LV) is depicted in Fig. 7 [78]. When injection is made by fast autosamplers and short insertion of a syringe needle into the injector, the injected liquid leaving the syringe needle forms a band. The band is shot into the packing material in the bottom of the liner inside the chamber without contact with the hot liner wall. The liquid is collected on a small piece of packing material (glass wool) where slow evaporation occurs. The solvent vapors expand into the liner, displacing and compressing the carrier gas. If the liner is a closed system for the time being (no accessible volumes from outside, such as the gas supply, the split and septum purge outlet), displacement results in a strong pressure increase and the carrier gas acts as a buffer to prevent sample vapors from

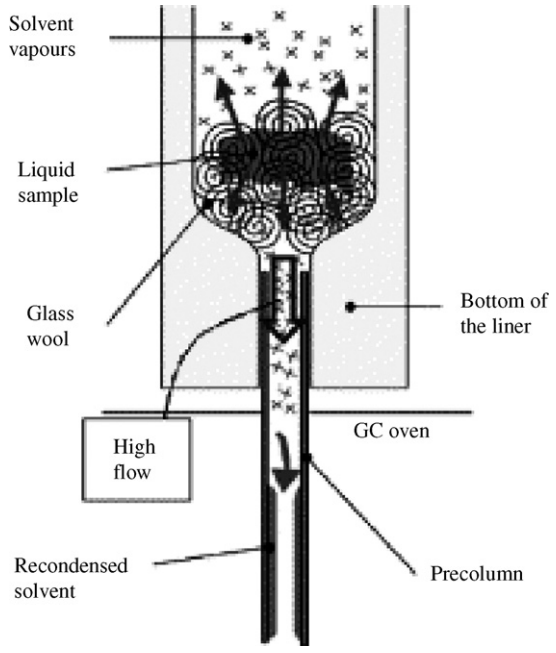

Fig. 7. Concurrent solvent recondensation (CSR) mechanism for large volume splitless injection (reprinted from ref. [78] with permission from the publisher).

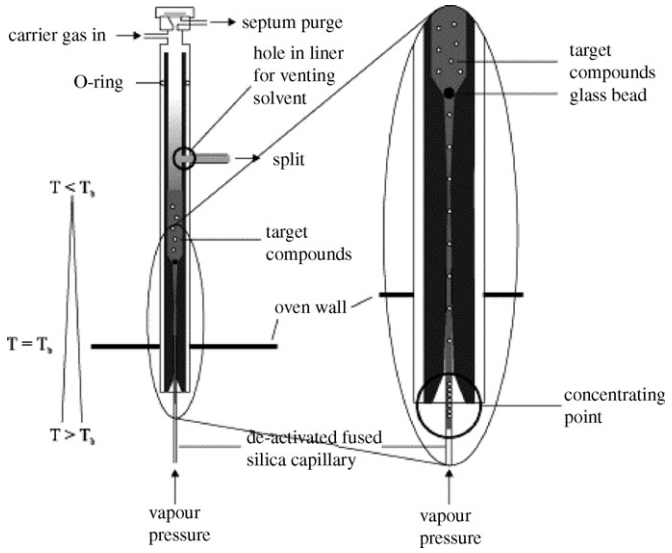

Fig. 8. At-column injector configuration (reprinted from ref. [56] with permission from the publisher).

escaping from the chamber. This "auto pressure surge" effect is self-regulated and drives the first solvent vapor to a retention gap. The solvent is recondensed at the beginning of the retention gap at a low oven temperature. This solvent recondensation causes a strong suction effect, increasing transfer of the sample to the column. A steady state is formed between the transfer rate of the vapor and the solvent evaporation rate in the injector. This concurrent solvent recondensation with evaporation enables almost unlimited sample volume to be injected. However, the solvent volume cannot exceed the capacity of the retention gap.

During the solvent evaporation, the temperature on the packing material is kept at the solvent boiling point. After the solvent evaporation is complete, the temperature returns to the thermostatted inlet temperature and high boiling compounds start vaporizing. Volatiles are reconcentrated by solvent trapping in the retention gap and high boiling compounds are refocused later on. No loss of high volatiles and discrimination of heavy compounds were observed in tests with hydrocarbons and polycyclic aromatic hydrocarbons (PAHs). This method requires an autosampler injection, a short syringe needle insertion, and the closure of the septum purge outlet during the splitless period, but not a complicated optimization, which makes CSR-LV a promising LVI method. Furthermore, there is a great potential that a laminar liner can be used in CRS-LV as an alternative to the deactivated glass wool for better inertness in the analysis of labile analytes [79].

4.3. At-column injection

Similarly to the above described CSR-LV technique, the at-column LVI method uses solvent vapor pressure to regulate the solvent evaporation and transfer rates. However, the solvent evaporation occurs at the top of a deactivated fused silica capillary (a retention gap) inserted into the at-column injector (see Fig. 8) [56,80]. The key factor in the at-column LVI method is a positive temperature gradient between the injector and the column oven during the injection. This temperature gradient is created by setting up the initial injector temperature below the solvent boiling point and the initial oven temperature above the solvent boiling point.

When the sample is injected, the liquid flows from the cool liner into the retention gap that is press-fit connected to the outlet of the liner. Some of the liquid reaches the retention gap, and the solvent starts to evaporate there. The created vapor pressure pushes the excess of the liquid in the retention gap back towards the liner. When the solvent vapor leaves the capillary (the pressure has dropped below the carrier gas pressure set on the system), the liquid in the retention gap evaporates and new liquid can flow in. This process is repeated until the last drops of solvent enter the retention gap. In the end, all solutes are concentrated at the top of the retention gap, and the injector and column oven are heated according to their temperature programs for a GC separation.

The at-column liner has a side hole for venting solvent and its location is high enough to retain the sample volume. In addition, the liner has a 1 mm diameter glass bead at the bottom that restricts liquid flow to the retention gap. For this reason, the at-column approach can use empty liners, which prevents decomposition of susceptible analytes. On the other hand, the solvent evaporation is slower due to the smaller surface area.

Only three parameters have to be optimized in the at-column approach; injector temperature, purge flow, and maximum sample volume. The liner used in the described at-column injector had 120 μl capacity for at once injection. For larger injection volume, repetitive or speed-controlled injections can be employed. The purge flow is not critical because the evaporation process is self-regulated. The optimum purge flow is 100–200 ml/min. The injector temperature should be close to the pressure corrected solvent boiling point, which can be calculated by the empirical Antoine equation. The evaluation tests show that the at-column LVI performs very well for the analysis of heavier molecules and thermally labile compounds. However, high volatiles can be lost in the solvent evaporation step, thus a lower boiling point solvent should be used if possible.

4.4. Through oven transfer adsorption desorption interface

Water is one of the most difficult solvents for GC analysis. Despite that, LVI of water in GC has been a hot topic because of its wide application potential. For example, aqueous samples including water or biological fluids could be analyzed directly for certain analytes without sample preparation. On-line reversed-phase LC–GC (RPLC–GC) could be more straightforward without solvent or phase change. Mostly, LVI of water has been accomplished through off- or on-line SPE or by PTV LVI with sorbent packed liners [1,15,16,18]. Venting of large amounts of water in PTV is a time-consuming process, which limits the maximum water injection volume. Moreover, the water evaporation requires higher injection temperature, which causes loss of analytes with solvent venting through the split outlet.

To improve the drawbacks in the PTV LVI of water, Grob and his co-workers suggested a "swing system" which includes

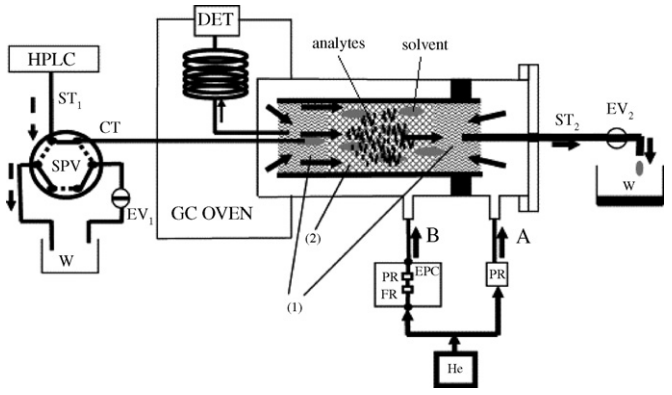

Fig. 9. Automated TOTAD interface during the transfer step (reprinted from ref. [82] with permission from the publisher). Symbols: (1) glass wool; (2) sorbent (Tenax TA); (SPV) six-port valve; (EV1 and EV2) electrovalves 1 and 2; (EPC) electronic pressure control; (PR) pressure regulator; (FR) flow regulator; (solid arrows) gas flow; (dotted arrows) liquid flow; (ST1) stainless steel tubing of 0.25 mm I.D. to transfer eluent from LC to GC; (ST2) stainless steel tubing of 1 mm I.D. to allow the exit of liquids and gases; (CT) silica capillary tubing between six-port valve and GC; (W) waste.

another PTV besides the regular PTV injector [53]. The sample is injected into the regular PTV; the solvent (water or methanol/water) is evaporated through the second PTV, which contains a sorbent for retention of highly volatile compounds, whereas high boiling compounds are retained in a retention gap. For the analyte elution, the gas flow is reversed by higher carrier gas applied to the extra PTV, which transfers the analytes from the sorbent and retention gap to the GC separation column.

Later, Villen and coworkers developed another system for LVI water introduction called "through oven transfer adsorption desorption (TOTAD)" interface [60,81]. The TOTAD interface is a modification of a PTV and its configuration is depicted in Fig. 9 [82]. The key features of the TOTAD interface include sample injection by a silica capillary tube (CT in Fig. 9) passing through the GC oven to the liner and solvent (water) elimination by a stainless steel tubing (ST) from the other side of the liner passing through the septum to a waste container. This solvent elimination to the waste container close to the septum (opposite direction to carrier gas flow direction in column) is made by another carrier gas flow (B in Fig. 9) in opposite direction to the GC column carrier gas flow (A in Fig. 9) direction. This new system enhances solvent elimination rate. A standard liner is packed with Tenax TA and plugged at both ends with glass wool. The sample is introduced to the interface by a six-port valve. Higher flow rate of B pushes the solution through the sorbent, the analytes are retained on the sorbent, and the solvent is vented to the waste through the ST tubing. The venting occurs in both modes, evaporation and solid-phase extraction, resulting in faster solvent elimination as compared to the regular PTV LVI.

During the transfer, the interface and oven temperatures are kept low (*e.g.* 80 °C for the interface and 40 °C for the oven when the solvent is water/methanol). After the transfer, the TOTAD interface is rapidly heated and the analytes are transferred to the column by thermal desorption. In this step, the carrier gas B should be off to transfer the analytes to the column. After the thermal desorption, the interface goes through the cleaning step, which involves heating up to a higher temperature.

The TOTAD interface was originally developed and mostly applied for polar solutions, such as for the direct introduction of water samples or fractions from RPLC [82–86]. Recently, Cortes et al. successfully extended the LVI application of this interface to other areas, such as the analysis of pesticides in vegetable extracts [87].

5. Applications

Applications of the LVI techniques are very diverse. Table 3 gives examples of LVI applications published in recent peer-reviewed papers (since 2000). Only the applications dealing with real samples were chosen and sorted according to the employed LVI technique, also providing information about the used solvent, injection volume, and sample matrix type.

The loop-type injector is an old classic LVI technique, originally designed for LC–GC coupling [1,16,88]. It is a simple technique: the sample present in the loop is transferred to a retention gap by the carrier gas with fully concurrent solvent evaporation. Therefore, its application range is limited to semi-volatiles and clean samples. However, because of its simplicity, this technique is still in use. For example, the loop-type interface was employed for the analysis of high boiling compounds, polybrominated diphenyl ethers (PBDEs) and PAHs in thoroughly cleaned samples [89,90].

The on-column LVI has been used for wide range of analytes from very volatiles, such as jet fuel volatile organic compounds and aroma compounds, to semi-volatiles, including for example polychlorinated biphenyls (PCBs) [91–93]. In addition, degradation of thermally labile compounds (such as pesticides captan, captafol, and folpet) was minimized by using the on-column LVI [94]. The tested sample matrices were relatively clean themselves (like air samples) or went through several clean-up procedures. For instance, the aroma compounds in carrots were extracted by dynamic headspace sampling and desorbed by methylene chloride, resulting in a relatively clean sample suitable for on-column LVI [92]. Kristenson et al. compared two LVI techniques, on-column and PTV, for the analysis of chloroanilines in soil samples and found that the PTV LVI was superior to on-column LVI in terms of robustness [95].

All PTV applications in Table 3 were conducted in solvent-split mode. It is clear that PTV is the predominant LVI technique. The application range of PTV is wide, but it is not suitable for high volatiles. The tested sample matrices were also diverse: from relatively clean samples, such as air and water, to very complex matrices, such as foods and sediments. The applications in Table 3 show that the PTV LVI facilitates modern sample preparation (extraction and pre-concentration) techniques, such as membrane-assisted solvent extraction (MASE) [96–99] or stir bar sorptive extraction followed by liquid desorption (SBSE-LD) [48,100–103] using the GC analysis without an evaporation step. These modern extraction techniques can handle only a limited sample size, so that the LVI is a needed requirement to reduce LODs, especially for the trace analysis. Wang et al. used the QuEChERS sample preparation method [104] for the

Table 3
Recent applications of the LVI techniques

LVI type	Analytes	Injected solvent	Injection volume (μl)	Matrix	Sample preparation	Ref.
Loop-type	PBDEs	Hexane	500	Air		[89]
	PAHs	Ethyl acetate	150	Sediments		[90]
On-column	PCBs and PAHs	Hexane/DCM	80/90	Soil		[93]
	Pesticides	Hexane/acetone	10–100	Air		[94]
	Jet fuel volatile organic compounds	Pentane	10	Human blood		[91]
	Chloroanilines	Hexane	100	Soil	PLE	[95]
	Haloanilines	Carbon disulfide	40	Water		[126]
	Phenols	Toluene	100	Water	In-tube SPME-LD	[127]
	Aroma compounds	DCM	15	Carrot	Dynamic headspace-LD	[92]
	Organic acids, PAHs and other organic compounds	Hexane (5% of ethyl acetate)	280/840	Air	On-line SFE–LC–GC	[128]
PTV	Dioxins	Toluene	10	Foodstuff		[129]
	PBDEs	Hexane	75	Air		[42]
	PBDEs	Hexane	20	Waste water, sediments and printed board circuit matrices	SBSE-LD	[48]
	PBDEs and PCBs	Hexane/DCM	20	Human adipose tissues		[130]
	PBDEs, PBBs and PCBs	Isooctane	70	Sediments		[49]
	PCBs	Cyclohexane	100	River water, white wine and apple juice	MASE	[96]
	PAHs	Hexane	50	Air		[131]
	Nitro-PAHs	Hexane/DCM	10			
	PAHs	DCM	70	Air		[50]
	PAHs	Toluene	50	Soil and sediments	PLE	[132]
	PAHs	Hexane/dodecane (1000:1)	720	Urban dust and diesel particulate matter	On-line LC–GC/MS	[44]
	Organochlorine contaminants	Acetonitrile/ethyl acetate	10/100	Municipal solid waste incineration fly ash		[133]
	Organochlorine contaminants	Isooctane	200	River water	SBSE-LD	[100]
	Organochlorine pesticides	Ethyl acetate	100	Water	SPE	[134]
	Organochlorine pesticides	Ethyl acetate	300	Water	On-line SPE-GC	[110]
	Organochlorine pesticides	Hexane	100	River water		[135]
	Organochlorine pesticides	Acetone/hexane	20	Air		[136]
	Multiclass pesticide residues	Ethyl acetate/cyclohexane	30	Vegetables/fruits		[41]
	Multiclass pesticide residues	Acetonitrile	20	Apple juice	QuEChERS method	[105]
	Multiclass pesticide residues	Acetone/hexane	200–400	Vegetables/fruits		[41]
	Triazines, organochlorine and organophosphorus pesticides	Cyclohexane	100	Waste water, wine, bacterial culture and orange juice	MASE	[97] [98]
	Organophosphorus pesticides	Ethyl acetate	10–40	Orange juice		[137]
		Acetonitrile	10			
	Triazine and organophosphorus pesticides	MtBE	200	Water	LLE/SPE	[106]
	Pyrethroid pesticides	Acetonitrile	20	Water	SBSE-LD	[103]
	Herbicides, organochlorine and organophosphorus pesticides, PAHs, PCBs, biocides, phthalates and alkylphenols	Ethyl acetate	20	Water	SBSE-LD	[101]
	Chloroanilines	Hexane/acetone	20	Soil	PLE	[95]
	Nonylphenols	Hexane	100	Foodstuff	HPLC-derivatization-GC	[138]
	Phenols	Ethyl acetate	100	Ground water	MASE	[99]

Table 3 (*Continued*)

LVI type	Analytes	Injected solvent	Injection volume (μl)	Matrix	Sample preparation	Ref.
	Phthalates	Ethyl acetate	20	Water	SBSE-LD	[102]
	Phthalates	Cyclohexane	50	Water	LLE	[108]
	Ethyl carbamate	Ethyl acetate	35	Wine	SPE	[139]
	Oxygen-related aldehydes	Hexane	40	Wine	SPE	[46]
	Aroma compounds	DCM/pentane	50	Wine		[140]
	Haloanisoles	DCM	40	Wine	SPE	[141]
	Off-flavor compounds (haloanisoles)	DCM	100	Water	Continuous LLE	[107]
	Organophosphate esters	MtBE	800	Air	Online dynamic microwave assisted extraction-SPE–GC	[111]
	Organophosphate esters	Hexane/MtBE	800	Air	On-line dynamic sonication assisted solvent	[112]
	Organophosphate esters	MtBE	150	Blood plasma	Miniaturized dynamic LLE	[109]
	Ropivacaine	Human plasma	50	Human plasma	Ultrafiltration	[142]
	Ropivacaine/bupivacaine	DCM	50	Human plasma	LLE	[143]
	Cannabionoids	Hexane	20	Human plasma	SPE	[144]
	Narcotics/stimulants	MtBE	20	Blood/saliva	SPE	[145]
	Trichloroacetic acid	Water	20–200	Water	PTV thermal decarboxylation	[114]
DSI/DMI	Multiclass pesticide residues	Acetonitrile	10	Eggs		[116]
	Multiclass pesticide residues	Acetonitrile	10	Fruit-based baby food	QuEChERS method	[69]
	Multiclass pesticide residues	Acetonitrile	10	Olives	QuEChERS method	[69,82,120]
	Multiclass pesticide residues	Ethyl acetate	10	Fruit-based baby food		[67]
	Multiclass pesticide residues	Ethyl acetate	10	Lettuce		[68]
	Multiclass pesticide residues	Acetonitrile	11	Fruit/vegetables		[117]
	Chlorophenoxy acid herbicides	Chloroform	10–20	Water	On-line derivatization	[122]
	Acrylamide	Acetonitrile	20	Various food matrices		[118]
	4-Nonylphenols	Chloroform	20	Sediments	PLE	[119]
	Alkylbenzenesulfonates	Chloroform	10–20	Sediments	On-line derivatization	[121]
	Naphthalenesulfonic acid isomers	Chloroform	10	Industrial effluents and river water	On-line derivatization	[123]
	Pharmaceutical residues	Chloroform	10	Water	On-line derivatization	[124]
At-column	Dioxins	Toluene	100	Human serum		[125]
TOTAD	Organophosphorus pesticides	Ethyl acetate	50	Vegetables		[87]
	Multiclass pesticide residues	Water/methanol	1000–2000	River water	Direct water injection	[83]
			2200	Olive oil	On-line RPLC–GC	[82]
	Multiclass pesticide residues	Methanol/water	400–2000			[84]
			3600			[85]
	Unsaponifiable compounds (tocopherols, etc.)	Methanol/water	1600	Edible oils	On-line RPLC–GC	[86]

DCM: dichloromethane; QuEChERS: quick, easy, cheap, effective, rugged, and safe; LD: liquid desorption; LLE: liquid–liquid extraction; MASE: membrane-assisted solvent extraction; MtBE: methyl *tert*-butyl ether; PAHs: polycyclic aromatic hydrocarbons; PBBs: polybrominated biphenyls; PBDEs: polybrominated diphenyl ethers; PCBs: polychlorinated biphenyls; PLE: pressurized liquid extraction; RPLC: reversed-phase liquid chromatography; SBSE: stir bar sorptive extraction; SFE: supercritical fluid extraction; SPME: solid-phase microextraction.

PTV-LVI-GC-MS multiclass, multiresidue analysis of 141 pesticides in apple juice [105]. PTV LVI of extracts of water samples prepared by simple liquid-liquid extraction (LLE) was successfully employed in the analysis of several different chemicals [106–109]. In addition, the PTV LVI technique was used as an interface in some on-line coupling applications between sample extractions or LC and GC [44,110–112]. In these on-line LC–GC applications, the injected volume was much larger than that in the other PTV LVI applications. Normal phase LC was used, thus the injected solvents to the GC were organic solvents with relatively low boiling points. Therefore, solvent venting was easy in that case and injection was conducted by multiple or continuous injection methods. To prevent loss of the analytes, dodecane or some other high-boiling solvent can be added to the LC eluent as a keeper [44].

Direct aqueous injection to GC poses a challenge because water deteriorates GC columns as mentioned earlier. In LVI-PTV solvent-split mode, water can be evaporated like other solvents, but it requires higher temperature and/or longer time. During water evaporation, the analytes should be retained in the liner. Teske et al. investigated suitable operating conditions to eliminate water completely in the LVI-PTV system. They found that addition of 2-propanol increased the water evaporation rate and that a carrier gas back-flush function helped to prevent water introduction into the GC column [113], as also shown in another application [114]. Tenax TA proved to be best liner packing material for direct injection of large volume of aqueous samples containing pesticides and nitroaromatics [115].

The DSI/DMI LVI has been mainly used in the analysis of pesticide residues in various food matrices [67–69,116–120], demonstrating the robustness of the DSI/DMI method for dirty matrix samples. Another interesting application of DSI/DMI is on-line derivatization of polar compounds in the injector [121–124]. This application provides a great advantage shortening the normally tedious and laborious derivatization process.

There are not many applications adapting at-column and splitless overflow (CRS-LV) techniques for real samples. The reason may be that they are relatively newer techniques. Kitamura et al. used the at-column LVI for dioxin analysis in human serum [125]. Dioxin concentration in human serum is very low, thus the standard method requires larger sample size and extremely high extract pre-concentration prior to the GC injection. The standard method injects 2 μl out of 20 μl final extract volume [125], whereas the at-column LVI technique could inject up to 100 μl, reducing the sample size of human serum from 25 g to 5 g.

As mentioned earlier, the TOTAD LVI technique facilitates direct large volume aqueous sample injection to the GC system [83]. Thus, it has been mostly employed for on-line RPLC–GC coupling [82,84–86]. For instance, edible oils can be directly injected to LC without any pre-clean up process other than filtration and analyzed for multiclass pesticide residues and some other components, including free sterols, tocopherols, squalene, and erythrodiol [86]. The examples in Table 3 show that the injection volumes in the TODAD were much larger than with the other LVI techniques, providing a suitable interface for on-line RPLC–GC system.

6. Summary and future trends

LVI is a prerequisite of modern GC analysis, especially when trace components have to be determined at very low levels. The most common LVI techniques are on-column and PTV-solvent split, and these techniques have been studied and modified to be more user-friendly and to overcome or reduce their limitations. Compared to the original design, installation of SVE and its electronic flow meter enable on-column LVI to be used practically. Availability of different packing materials, liners, and systematic PTV parameter optimization broaden the scope of PTV LVI applications. On-column LVI is superior for high volatiles and thermally labile compounds, whereas PTV LVI is beneficial in the analysis of dirty matrix samples. New LVI techniques have been developed during the past decade. Among them, we discussed four promising LVI techniques in this paper, modified PTV techniques (DSI/DMI and TOTAD) and overflow techniques (splitless overflow and at-column).

The current trends in analytical chemistry include simplification of sample preparation, adaptation of environmentally friendly methods, and automation or on-line coupling of the analytical procedures. For example, selective extraction methods (such as SPE, MASE, SPME, SBSE, or in-tube SPME) have been replacing the classical exhaustive extraction methods, such as Soxhlet. The extracts from these modern extraction methods can be injected directly into the GC system in off- or on-line in LVI without a pre-concentration step. This simplified sample preparation makes routine analysis of a large number of samples fast and easy. Also, coupling of LC and GC is a very powerful system for substantial time saving in sample preparation and better reproducibility. The LC offers high sample capacity and wide range of separation mechanisms, thus it can be utilized in selective clean-up. The GC provides high separation efficiency and a variety of detection methods. In addition, the closed system reduces error potentially occurring in off-line sample preparation. To transfer the LC fractions to the GC system on-line, a high-capacity LVI is required. A recently developed TOTAD injector has a large capacity for polar solvents and has been successfully adapted in on-line RPLC–GC. Recent interest in comprehensive two-dimensional LC × GC separations, in which all fractions eluting from LC are introduced into the GC system, will probably be a driving force in future developments in on-line RPLC–GC coupling.

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